

Attorney Docket No.: DEX0531US.NP
Inventors: Wolfert et al.
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This listing of the claims will replace all prior versions and listings of claims in the application:

Listing of the claims:

Claim 1 (original): A method for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising:

- (a) contacting an immobilized binder, which specifically binds Lp-PLA2, with the sample;
- (b) washing the immobilized binder to remove an enzymatically active unbound material or an interfering substance(s);
- (c) contacting the bound Lp-PLA2 with a substrate converted to a detectable product in the presence of Lp-PLA2; and
- (d) measuring detectable product indicative of enzymatically active Lp-PLA2 in the sample.

Claim 2 (currently amended): The method of claim 1, wherein the sample is a serum sample, a plasma sample, or an EDTA treated plasma sample ~~or an EDTA treated serum sample~~.

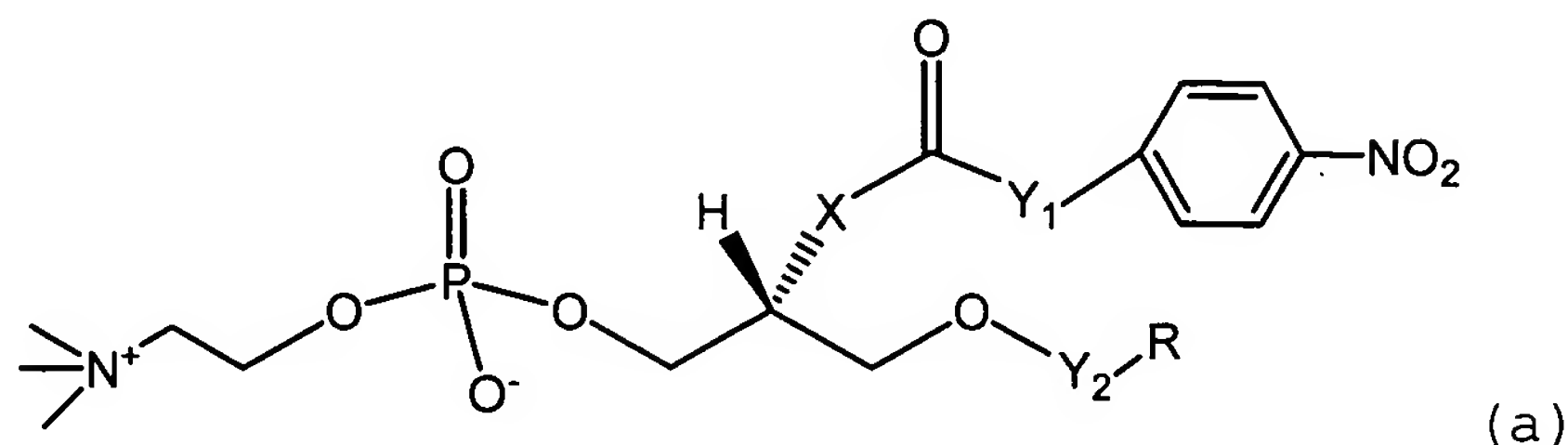
Claim 3 (original): The method of claim 1, wherein the immobilized binder is an antibody.

Claim 4 (original): The method of claim 3, wherein the antibody is a monoclonal antibody, a phage display antibody, or a polyclonal antibody.

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Claim 5-7 (canceled)

Claim 8 (original): The method of claim 1, wherein the substrate is selected from the group consisting of



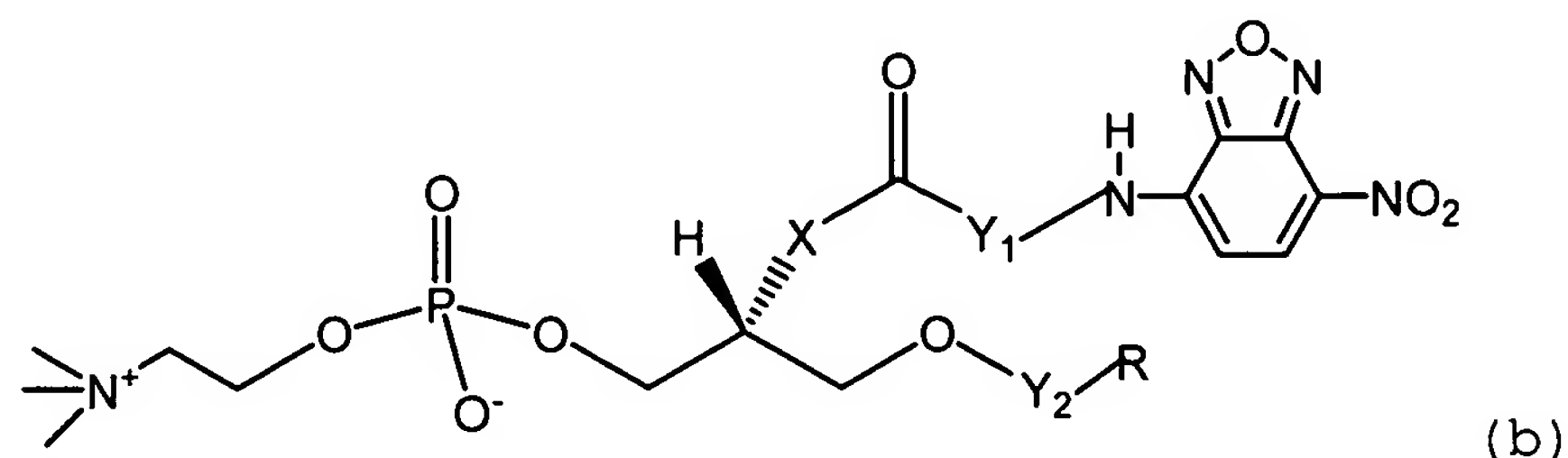
wherein,

X is selected from the group consisting of O, S, and -O(CO)-;

R is selected from the group consisting of (CH₂)₄CH₃, (CH₂)₆CH₃, (CH₂)₈CH₃, (CH₂)₁₀CH₃, (CH₂)₁₂CH₃, (CH₂)₁₄CH₃, and (CH₂)₇CH=CH(CH₂)₂CH₃;

Y₁ is selected from the group consisting of (CO)₁₋₂ and (CH₂)₂₋₇; and

Y₂ is selected from the group consisting of CO and CH₂;



wherein,

X is selected from the group consisting of O, S, and -O(CO)-;

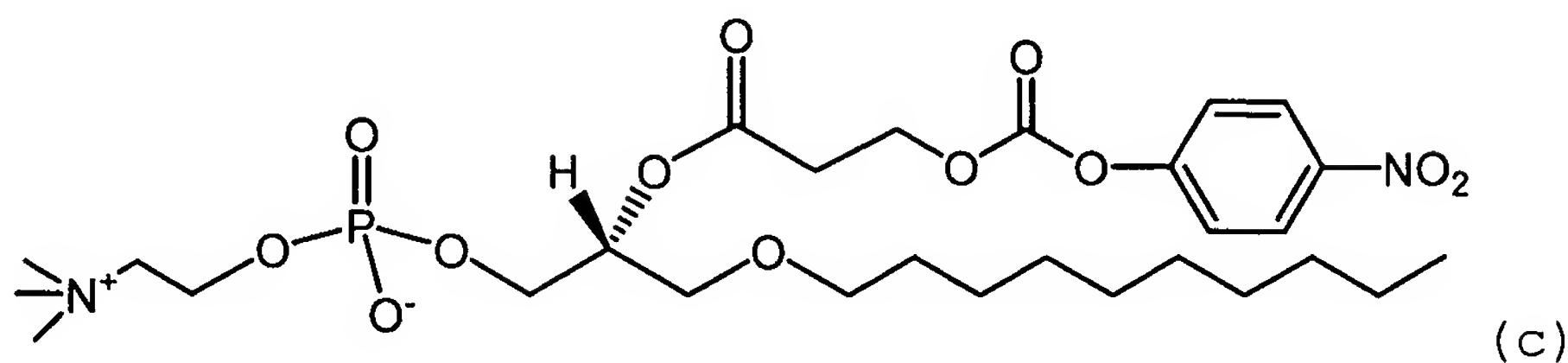
R is selected from the group consisting of (CH₂)₄CH₃, (CH₂)₆CH₃, (CH₂)₈CH₃, (CH₂)₁₀CH₃, (CH₂)₁₂CH₃, (CH₂)₁₄CH₃ and (CH₂)₇CH=CH(CH₂)₂CH₃;

Y₁ is selected from the group consisting of (CO)₁₋₂ and (CH₂)₂₋₇;

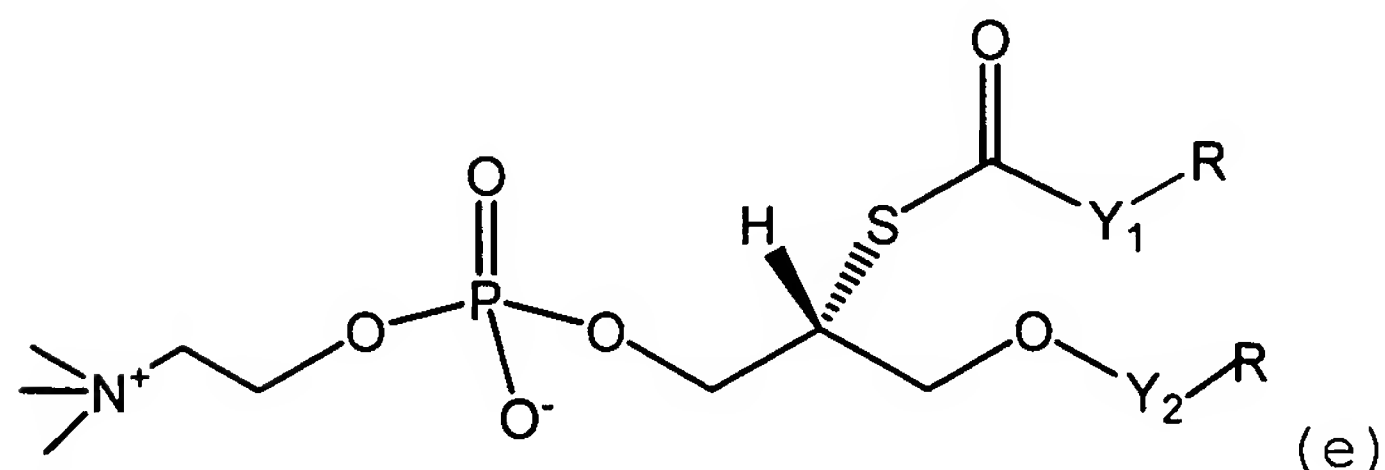
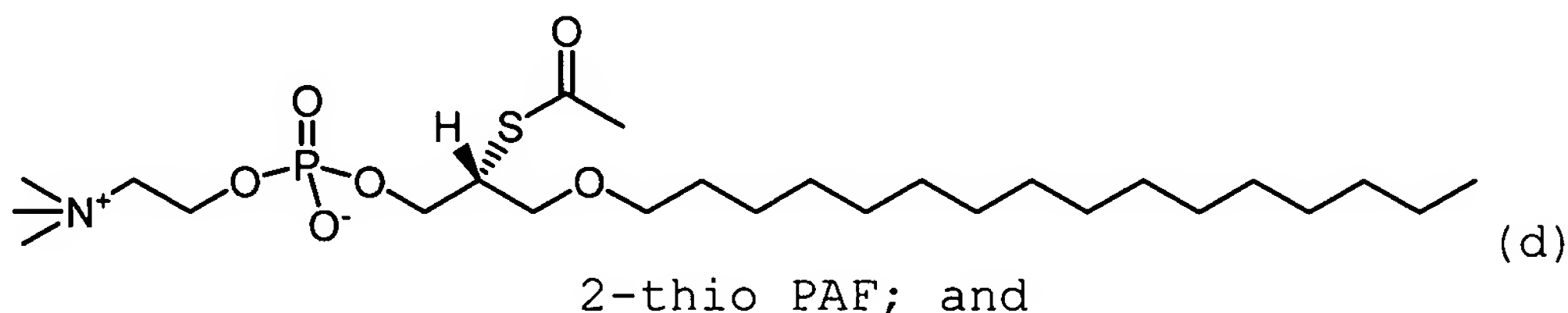
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and

Y_2 is selected from the group consisting of CO and CH_2 ;



1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine (MNP);



wherein

X is selected from the group consisting of O, S, and $-O(CO)-$;

R is selected from the group consisting of $(CH_2)_4CH_3$, $(CH_2)_6CH_3$, $(CH_2)_8CH_3$, $(CH_2)_{10}CH_3$, $(CH_2)_{12}CH_3$, $(CH_2)_{14}CH_3$, and $(CH_2)_7CH=CH(CH_2)_2CH_3$;

Y_1 is selected from the group consisting of $(CO)_{1-2}$ and $(CH_2)_{2-7}$;
 and

Y_2 is selected from the group consisting of CO or CH_2 .

Claim 9 (original): The method of claim 8 where in the substrate is an oxidized derivative of (a), (b), (c), (d) or (e).

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Claim 10-11 (canceled)

Claim 12 (currently amended): The method of claim 1 which further ~~comprising~~ comprises comparing the measured detectable product of step (d) to detectable product in a control comprising an enzymatically active Lp-PLA2 standard.

Claim 13-16 (canceled)

Claim 17 (currently amended): A method for detecting vascular disease in an individual comprising utilizing the method of ~~claim 16~~ claim 1 to determine the individual's Lp-PLA2 activity in a sample wherein increased activity of Lp-PLA2 in the sample is indicative of vascular disease.

Claim 18 (canceled)

Claim 19 (currently amended): A method for selecting an individual for therapy to treat vascular disease comprising utilizing the method of ~~claim 16~~ claim 1 to determine the individual's Lp-PLA2 activity in a sample wherein increased activity of Lp-PLA2 in the sample is indicative of an individual who will benefit from therapy to treat vascular disease.

Claim 20-21 (canceled)

Claim 22 (currently amended): A method for monitoring an ~~individuals~~ individual's response to therapy to treat vascular

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disease comprising utilizing the method of ~~claim 16~~ claim 1 to determine the individual's Lp-PLA2 activity in a sample wherein decreased activity of Lp-PLA2 in the sample is indicative of an individual who is responding favorably to therapy to treat vascular disease.

Claim 23-24 (canceled)

Claim 25 (currently amended) A method for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising:

- (a) contacting ~~an binder~~ a binder, which specifically binds Lp-PLA2, with the sample to form a binder-Lp-PLA2 complex;
- (b) immobilizing the binder-Lp-PLA2 complex;
- (c) washing the immobilized binder-Lp-PLA2 complex to remove an enzymatically active unbound material or an interfering substance(s);
- (d) contacting the immobilized bound Lp-PLA2 with a substrate converted to a detectable product in the presence of Lp-PLA2; and
- (e) measuring detectable product indicative of enzymatically active Lp-PLA2 in the sample.

Claim 26 (original): The method of claim 25, wherein the sample is a serum sample, a plasma sample or an EDTA treated plasma sample.

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Claim 27 (original): The method of claim 25, wherein the binder is an antibody.

Claim 28-29 (canceled)

Claim 30 (currently amended): The method of claim 25 wherein the binder-Lp-PLA2 complex is immobilized by binding to an immobilized compound, said immobilized compound comprising an antibody, protein or compound capable of binding the binder-Lp-PLA2 complex.

Claim 31-34 (canceled)

Claim 35 (original): The method of claim 25 wherein the binder is conjugated to an immobilizing agent.

Claim 36 (original): The method of claim 35, wherein the binder conjugated to an immobilizing agent is an antibody.

Claim 37-38 (canceled)

Claim 39 (original): The method of claim 35 wherein the immobilizing agent is an antibody, protein or compound capable of binding an immobilized compound.

Claim 40-41 (canceled)

Claim 42 (original): The method of claim 35 wherein the immobilizing agent is biotin.

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Claim 43 (original): The method of claim 35 wherein the immobilizing agent, conjugated to the binder-Lp-PLA2 complex, binds to an immobilized compound.

Claim 44 (original) The method of claim 43 wherein the immobilized compound is bound to a multi-well plate, a magnetic bead, or a latex bead.

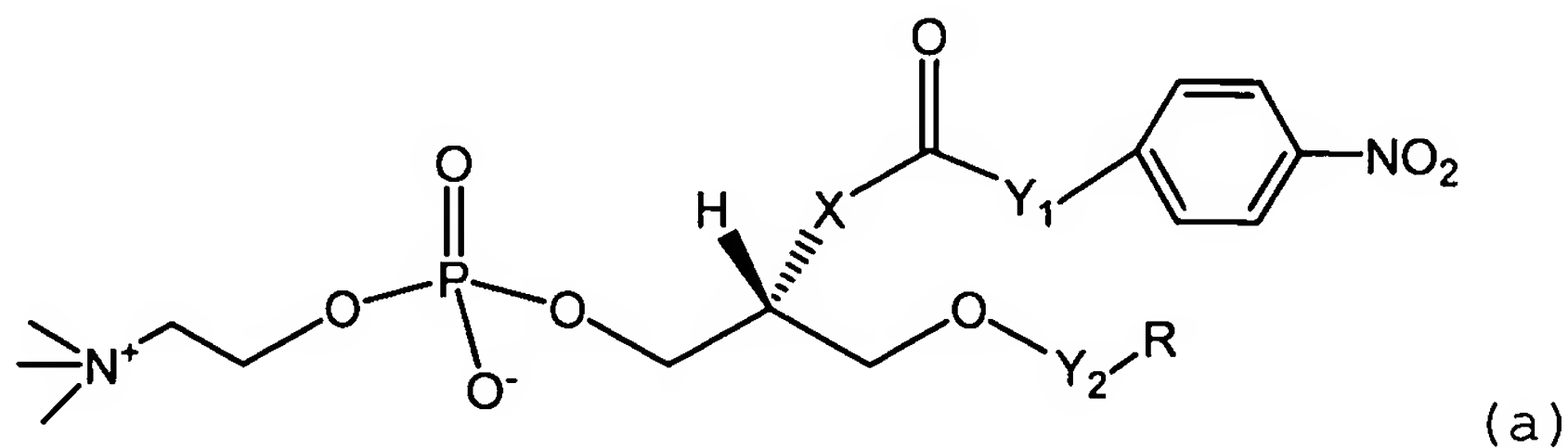
Claim 45 (original) The method of claim 44 wherein the bound compound is an antibody, protein or compound capable of binding the conjugated immobilizing agent.

Claim 46-47 (canceled)

Claim 48 (original): The method of claim 45 wherein the bound substance is streptavidin.

Claim 49-50 (canceled)

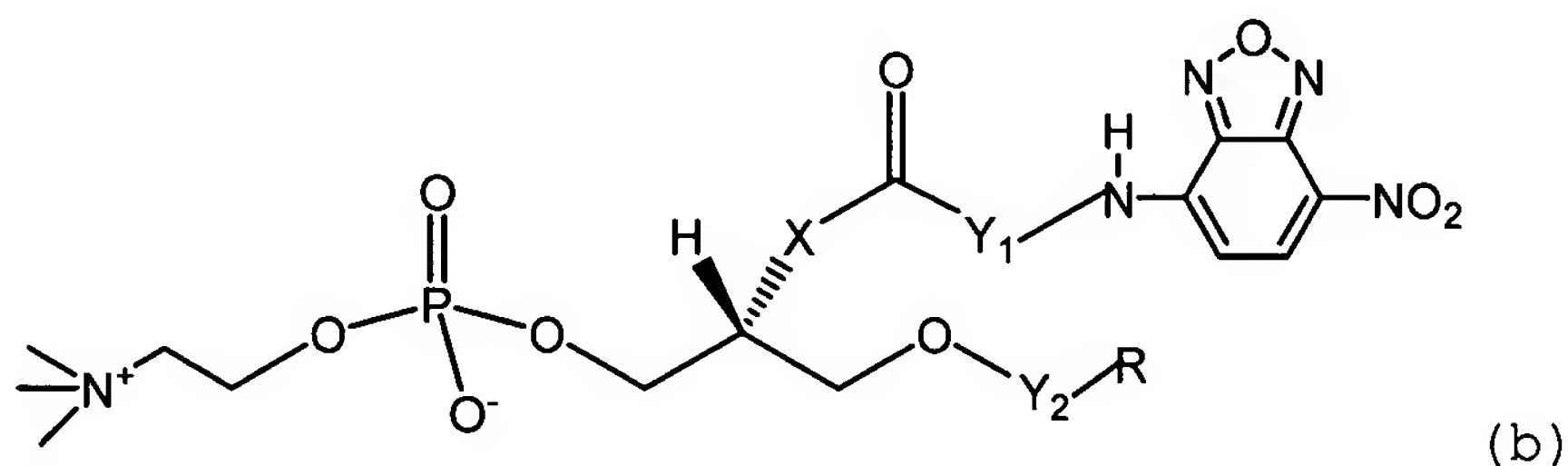
Claim 51 (original): The method of claim 25, wherein the substrate is selected from the group consisting of



wherein,

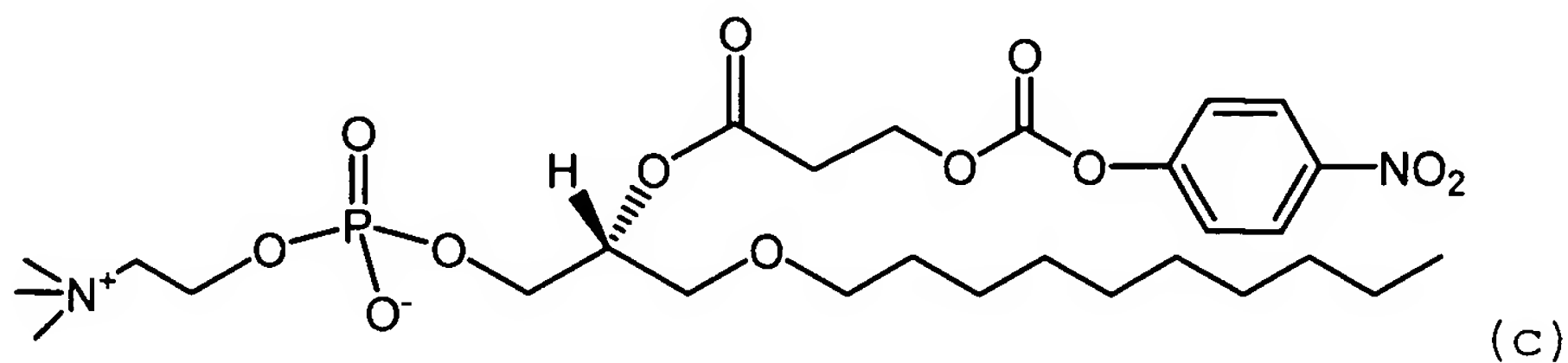
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X is selected from the group consisting of O, S, and -O(CO)-;
 R is selected from the group consisting of (CH₂)₄CH₃, (CH₂)₆CH₃,
 (CH₂)₈CH₃, (CH₂)₁₀CH₃, (CH₂)₁₂CH₃, (CH₂)₁₄CH₃, and
 (CH₂)₇CH=CH(CH₂)₂CH₃;
 Y₁ is selected from the group consisting of (CO)₁₋₂ and (CH₂)₂₋₇;
 and
 Y₂ is selected from the group consisting of CO and CH₂;



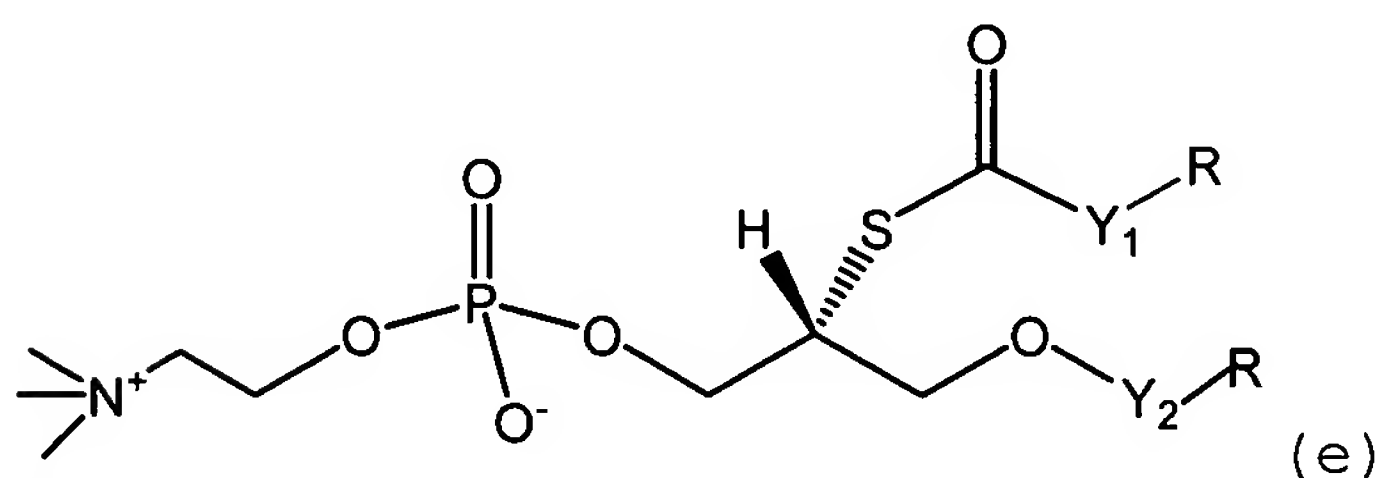
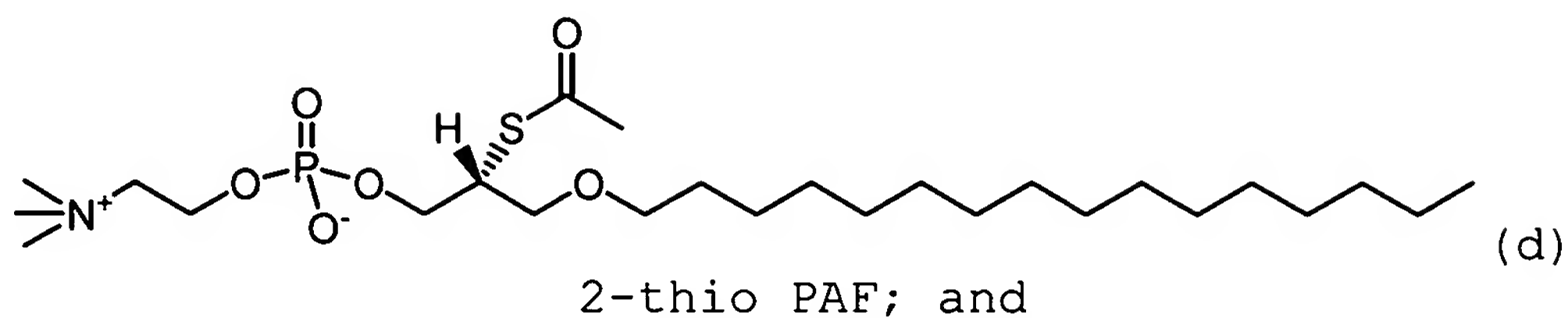
wherein,

X is selected from the group consisting of O, S, and -O(CO)-;
 R is selected from the group consisting of (CH₂)₄CH₃, (CH₂)₆CH₃,
 (CH₂)₈CH₃, (CH₂)₁₀CH₃, (CH₂)₁₂CH₃, (CH₂)₁₄CH₃ and (CH₂)₇CH=CH(CH₂)₂CH₃;
 Y₁ is selected from the group consisting of (CO)₁₋₂ and (CH₂)₂₋₇;
 and
 Y₂ is selected from the group consisting of CO and CH₂;



1-myristoyl-2-(4-nitrophensuccinyl) phosphatidylcholine (MNP);

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wherein

X is selected from the group consisting of O, S, and -O(CO)-;
 R is selected from the group consisting of (CH₂)₄CH₃, (CH₂)₆CH₃,
 (CH₂)₈CH₃, (CH₂)₁₀CH₃, (CH₂)₁₂CH₃, (CH₂)₁₄CH₃, and
 (CH₂)₇CH=CH(CH₂)₂CH₃;

Y₁ is selected from the group consisting of (CO)₁₋₂ and (CH₂)₂₋₇;
 and

Y₂ is selected from the group consisting of CO or CH₂.

Claim 52 (original): The method of claim 51 where in the
 substrate is an oxidized derivative of (a), (b), (c), (d) or (e).

Claim 53-54 (canceled)

Claim 55 (original): The method of claim 25 further comprising
 comparing the measured detectable product of step (e) to
 detectable product in a control comprising an enzymatically
 active Lp-PLA2 standard.

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Claim 56-58 (canceled)

Claim 59 (currently amended): A method for detecting vascular disease in an individual comprising utilizing the method of ~~claim 58~~ claim 55 to determine the individual's Lp-PLA2 activity in a sample wherein increased activity of Lp-PLA2 in the sample is indicative of vascular disease.

Claim 60 (canceled)

Claim 61 (currently amended): A method for selecting an individual for therapy to treat vascular disease comprising utilizing the method of ~~claim 58~~ claim 55 to determine the individual's Lp-PLA2 activity in a sample wherein increased activity of Lp-PLA2 in the sample is indicative of an individual who will benefit from therapy to treat vascular disease.

Claim 62-63 (canceled)

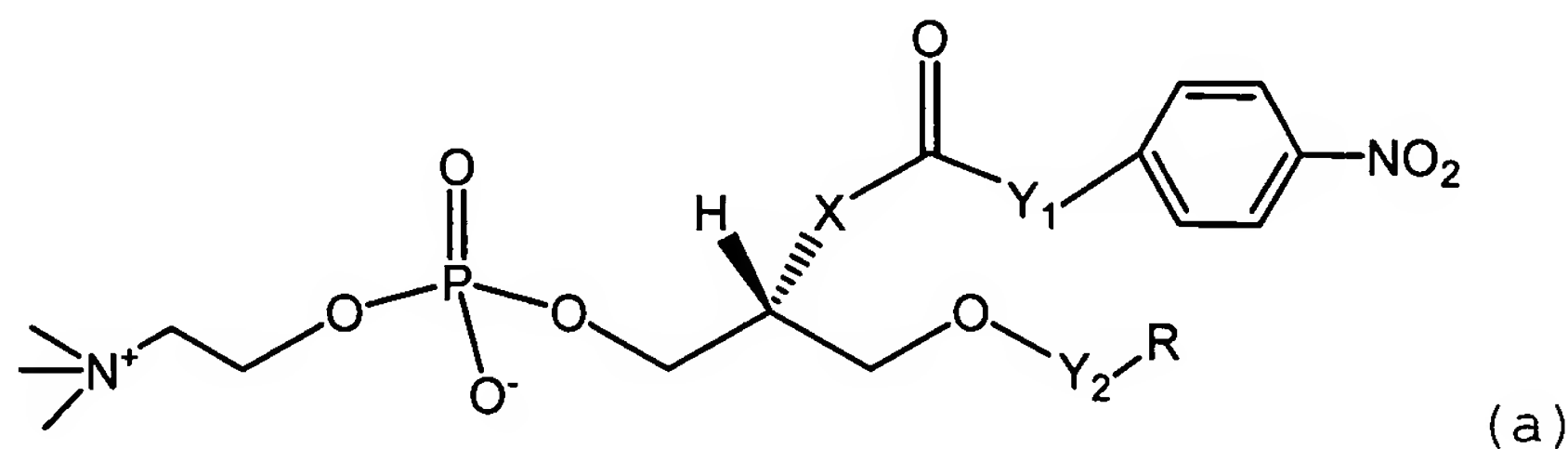
Claim 64 (currently amended): A method for monitoring an ~~individuals~~ individual's response to therapy to treat vascular disease comprising utilizing the method of ~~claim 58~~ claim 55 to determine the individual's Lp-PLA2 activity in a sample wherein decreased activity of Lp-PLA2 in the sample is indicative of an individual who is responding favorably to therapy to treat vascular disease.

Claim 65-66 (canceled)

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Claim 67 (original): A kit for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising a binder which specifically binds Lp-PLA2 and a substrate converted to a detectable product in the presence of Lp-PLA2.

Claim 68 (original): The kit of claim 67 wherein the substrate is selected from the group consisting of



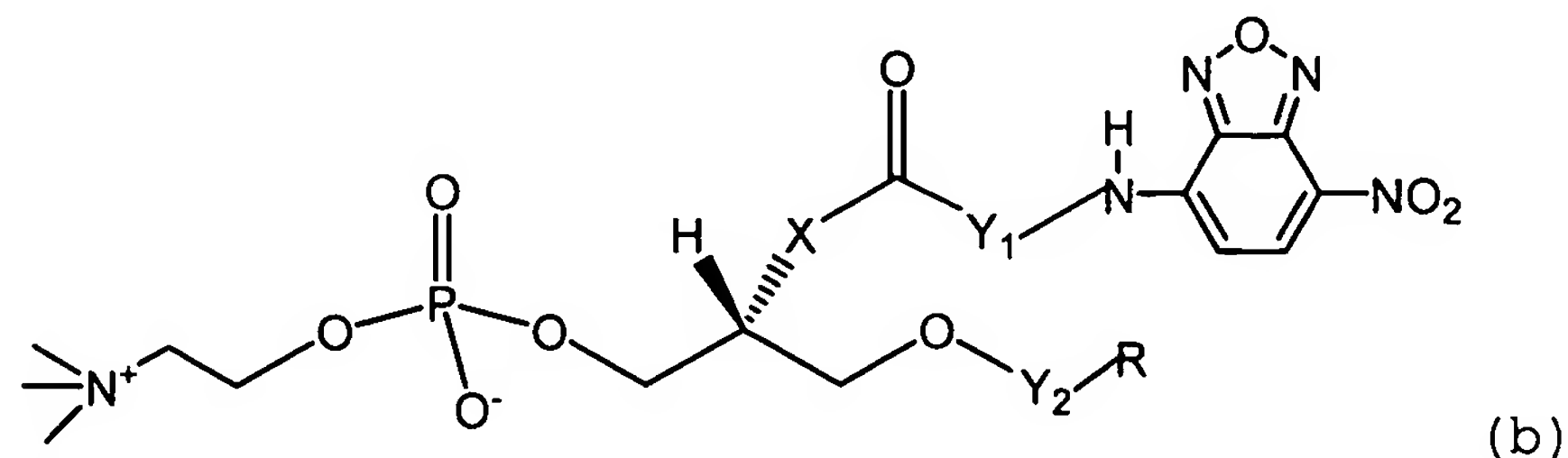
wherein,

X is selected from the group consisting of O, S, and -O(CO)-;

R is selected from the group consisting of (CH₂)₄CH₃, (CH₂)₆CH₃, (CH₂)₈CH₃, (CH₂)₁₀CH₃, (CH₂)₁₂CH₃, (CH₂)₁₄CH₃, and (CH₂)₇CH=CH(CH₂)₂CH₃;

Y₁ is selected from the group consisting of (CO)₁₋₂ and (CH₂)₂₋₇; and

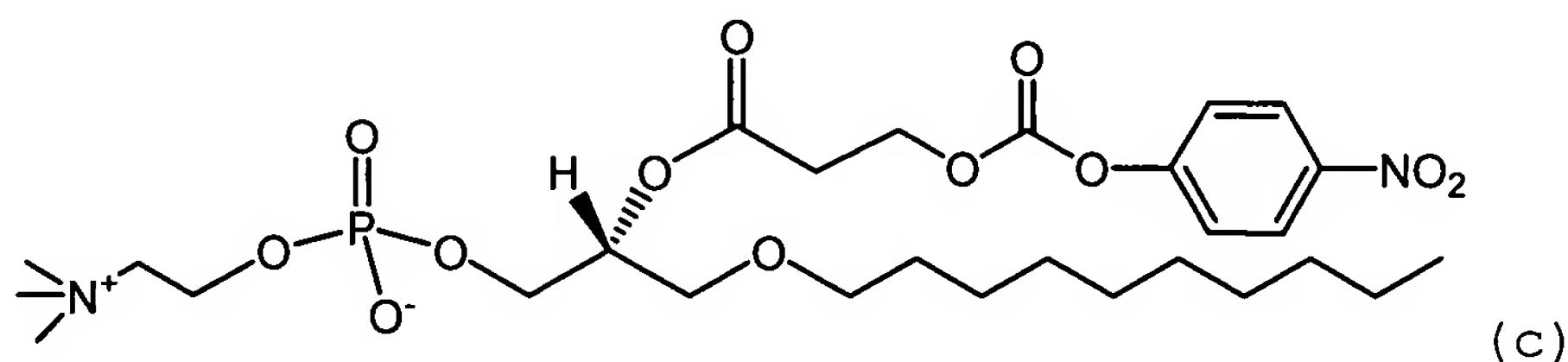
Y₂ is selected from the group consisting of CO and CH₂;



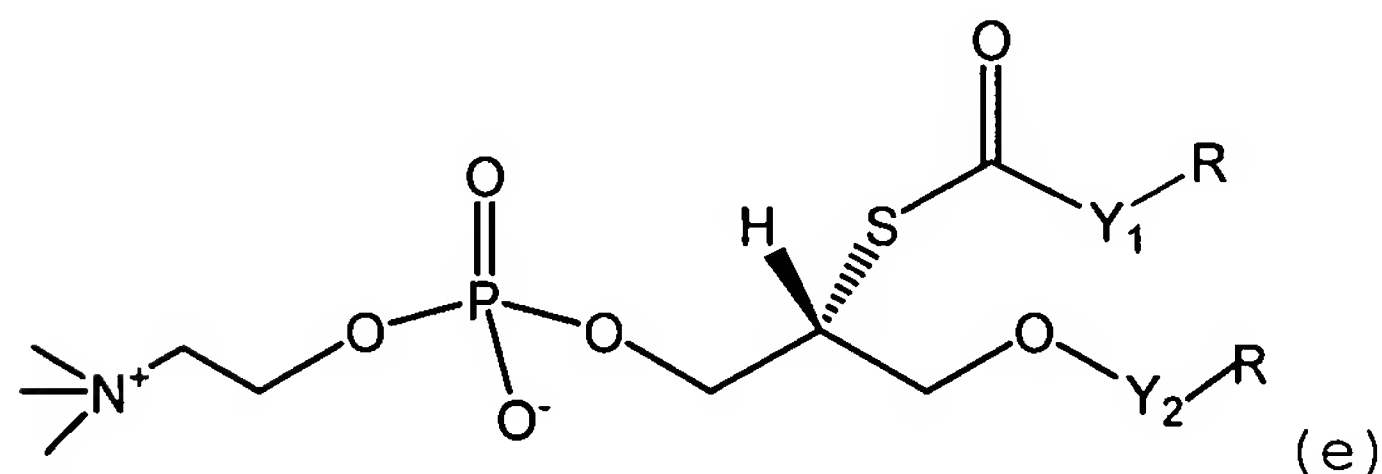
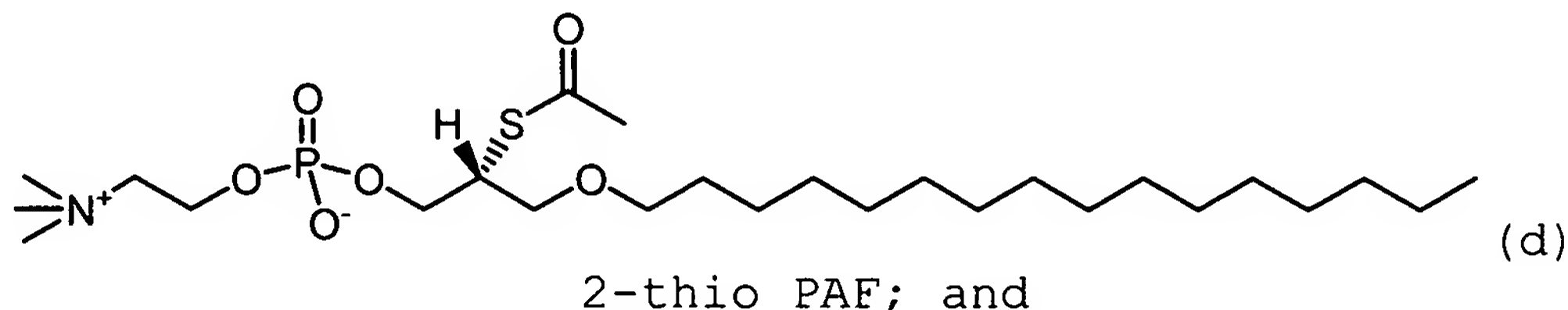
wherein,

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X is selected from the group consisting of O, S, and $-O(CO)-$;
 R is selected from the group consisting of $(CH_2)_4CH_3$, $(CH_2)_6CH_3$,
 $(CH_2)_8CH_3$, $(CH_2)_{10}CH_3$, $(CH_2)_{12}CH_3$, $(CH_2)_{14}CH_3$ and $(CH_2)_7CH=CH(CH_2)_2CH_3$;
 Y_1 is selected from the group consisting of $(CO)_{1-2}$ and $(CH_2)_{2-7}$;
 and
 Y_2 is selected from the group consisting of CO and CH_2 ;



1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine (MNP);



wherein

X is selected from the group consisting of O, S, and $-O(CO)-$;
 R is selected from the group consisting of $(CH_2)_4CH_3$, $(CH_2)_6CH_3$,
 $(CH_2)_8CH_3$, $(CH_2)_{10}CH_3$, $(CH_2)_{12}CH_3$, $(CH_2)_{14}CH_3$, and
 $(CH_2)_7CH=CH(CH_2)_2CH_3$;
 Y_1 is selected from the group consisting of $(CO)_{1-2}$ and $(CH_2)_{2-7}$;
 and

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Y₂ is selected from the group consisting of CO or CH₂.

Claim 69 (original): The kit of claim 68 wherein the substrate is an oxidized derivative of (a), (b), (c), (d) or (e).

Claim 70 (original): The kit of claim 67 further comprising an enzymatically active Lp-PLA2 standard.

Claim 71-72 (canceled)

Claim 73 (original): A method for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising:

- (a) incubating the sample with a compound which reduces active thiol(s) in the sample;
- (b) contacting the incubated sample with a substrate converted to a free thiol product in the presence of enzymatically active Lp-PLA2; and
- (c) measuring free thiol product indicative of enzymatically active Lp-PLA2 in the sample.

Claim 74 (original): The method of claim 73, wherein the sample is a serum sample, a plasma sample or an EDTA treated plasma sample.

Claim 75 (canceled)

Claim 76 (currently amended): The method of claim 73 wherein the sample is incubated at room temperature or at 37°C.

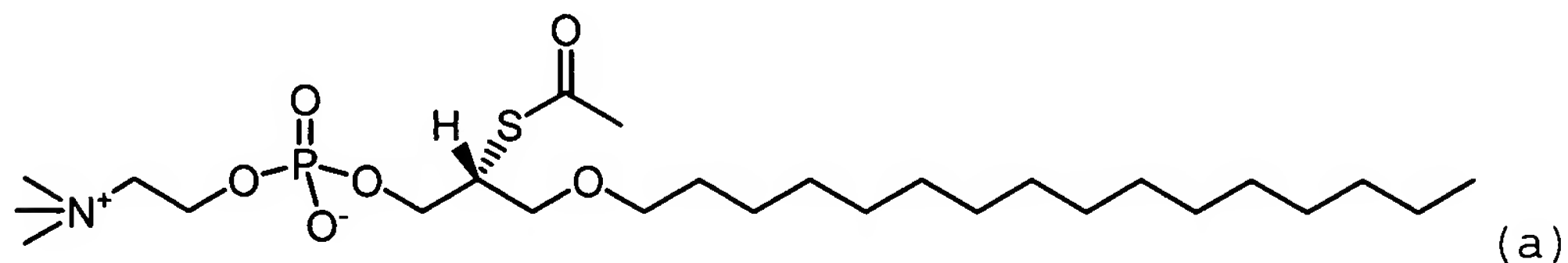
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Claim 77 (canceled)

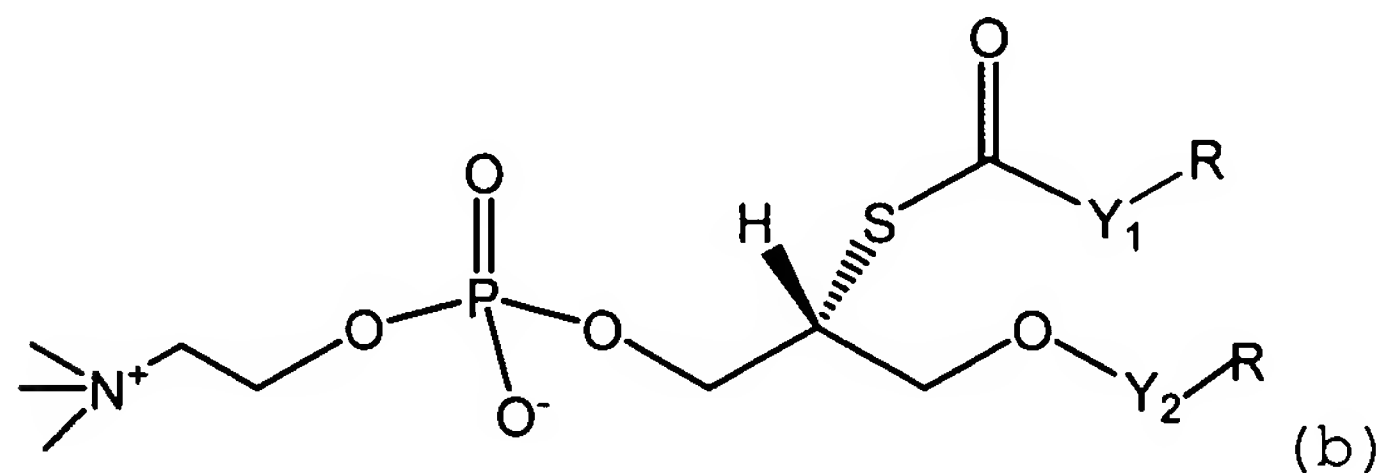
Claim 78 (original): The method of claim 73 wherein the sample is incubated from about 2 to about 120 minutes.

Claim 79 (canceled)

Claim 80 (original): The method of claim 73 wherein the substrate is selected from the group consisting of



2-thio PAF; and



wherein,

R is selected from the group consisting of $(\text{CH}_2)_4\text{CH}_3$, $(\text{CH}_2)_6\text{CH}_3$, $(\text{CH}_2)_8\text{CH}_3$, $(\text{CH}_2)_{10}\text{CH}_3$, $(\text{CH}_2)_{12}\text{CH}_3$, $(\text{CH}_2)_{14}\text{CH}_3$ and $(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}_3$;

Y_1 is selected from the group consisting of $(\text{CO})_{1-2}$ and $(\text{CH}_2)_{2-7}$;

and

Y_2 is selected from the group consisting of CO and CH_2 .

Claim 81 (original): The method of claim 80 where in the

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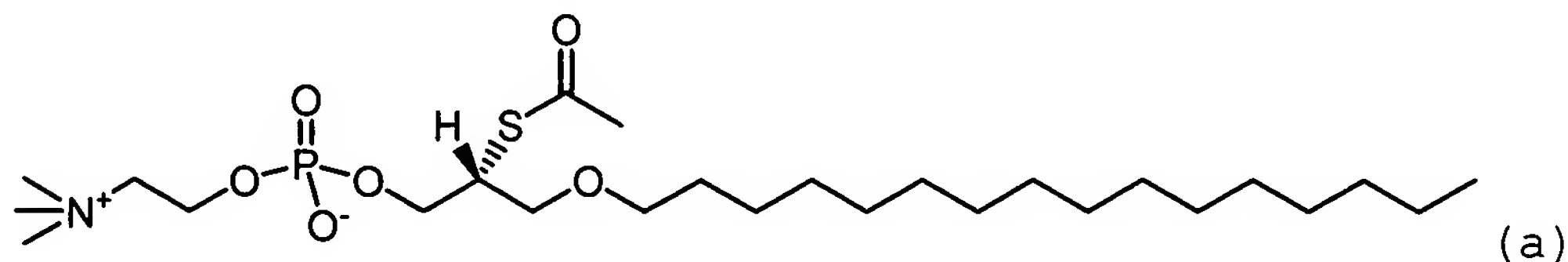
substrate is an oxidized derivative of (a) or (b).

Claim 82 (original): The method of claim 73 further comprising comparing measured free thiol product of step (c) to free thiol product in a control comprising an enzymatically active Lp-PLA2 standard.

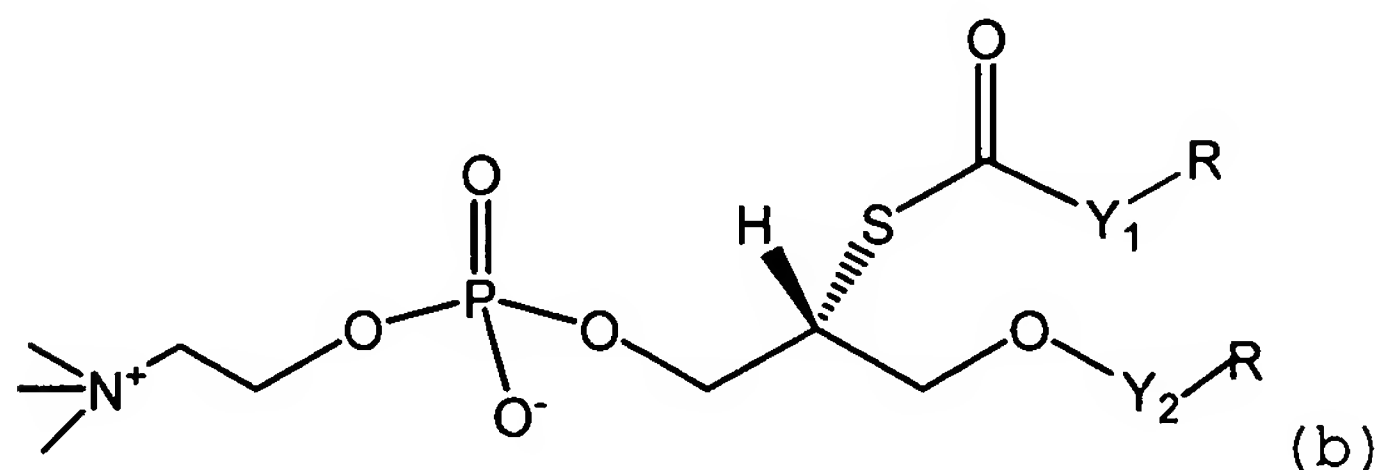
Claim 83-85 (canceled)

Claim 86 (original): A kit for measuring enzymatically active Lipoprotein-associated Phospholipase A2 (Lp-PLA2) in a sample comprising a compound which reduces active thiol(s) and a substrate converted to a detectable product in the presence of Lp-PLA2.

Claim 87 (original): The kit of claim 86 wherein the substrate is selected from the group consisting of



2-thio PAF; and



wherein,

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R is selected from the group consisting of $(\text{CH}_2)_4\text{CH}_3$, $(\text{CH}_2)_6\text{CH}_3$, $(\text{CH}_2)_8\text{CH}_3$, $(\text{CH}_2)_{10}\text{CH}_3$, $(\text{CH}_2)_{12}\text{CH}_3$, $(\text{CH}_2)_{14}\text{CH}_3$ and $(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}_3$;

Y_1 is selected from the group consisting of $(\text{CO})_{1-2}$ and $(\text{CH}_2)_{2-7}$;
and

Y_2 is selected from the group consisting of CO and CH_2 .

Claim 88 (original): The kit of claim 87 where in the substrate is an oxidized derivative of (a) or (b).

Claim 89 (original): The kit of claim 86 further comprising an enzymatically active Lp-PLA2 standard.

Claim 90-91 (canceled)